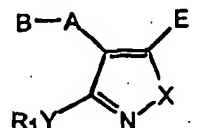
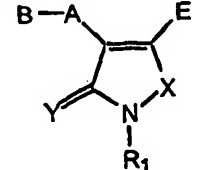
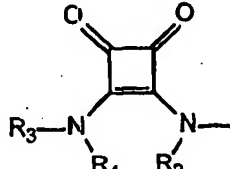




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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; vertical-align: top; padding: 5px;"> <p>(21) International Application Number: PCT/DK97/00426</p> <p>(22) International Filing Date: 3 October 1997 (03.10.97)</p> <p>(30) Priority Data: 1092/96 4 October 1996 (04.10.96) DK</p> <p>(71) Applicant (for all designated States except US): H. LUNDBECK A/S [DK/DK]; Ottiliavej 9, DK-2500 Copenhagen-Valby (DK).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): BANG-ANDERSEN, Benny [DK/DK]; Nørrebrogade 48, 4.tv, DK-2200 Copenhagen N (DK). BØGESØ, Klaus, Peter [DK/DK]; Hørsholm Park 16, 2.tv, DK-2970 Hørsholm (DK). KROGSGAARD-LARSEN, Povl [DK/DK]; Elmevej 25, DK-3450 Allerød (DK). MOLTZEN LENZ, Sibylle [DK/DK]; Toftekærvej 29, DK-2820 Gentofte (DK).</p> <p>(74) Common Representative: H. LUNDBECK A/S; John Meidahl Petersen, Ottiliavej 9, DK-2500 Copenhagen-Valby (DK).</p> </td> <td style="width: 50%; vertical-align: top; padding: 5px;"> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p> </td> </tr> </table>			<p>(21) International Application Number: PCT/DK97/00426</p> <p>(22) International Filing Date: 3 October 1997 (03.10.97)</p> <p>(30) Priority Data: 1092/96 4 October 1996 (04.10.96) DK</p> <p>(71) Applicant (for all designated States except US): H. LUNDBECK A/S [DK/DK]; Ottiliavej 9, DK-2500 Copenhagen-Valby (DK).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): BANG-ANDERSEN, Benny [DK/DK]; Nørrebrogade 48, 4.tv, DK-2200 Copenhagen N (DK). BØGESØ, Klaus, Peter [DK/DK]; Hørsholm Park 16, 2.tv, DK-2970 Hørsholm (DK). KROGSGAARD-LARSEN, Povl [DK/DK]; Elmevej 25, DK-3450 Allerød (DK). MOLTZEN LENZ, Sibylle [DK/DK]; Toftekærvej 29, DK-2820 Gentofte (DK).</p> <p>(74) Common Representative: H. LUNDBECK A/S; John Meidahl Petersen, Ottiliavej 9, DK-2500 Copenhagen-Valby (DK).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>
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<p>(54) Title: 3-ALKOXYISOXAZOL-4-YL-SUBSTITUTED 2-AMINO CARBOXYLIC ACID COMPOUNDS</p> <div style="display: flex; justify-content: space-around; align-items: center; margin-top: 20px;"> <div style="text-align: center;">  <p>(I)</p> </div> <div style="text-align: center;">  <p>(II)</p> </div> </div> <div style="text-align: center; margin-top: 20px;">  <p>(III)</p> </div> <p>(57) Abstract</p> <p>(3-Alkoxyisoxazol-4-yl)-substituted 2-amino carboxylic acid derivatives and sulfur analogues thereof having general Formula (I) or (II), wherein R₁ is hydrogen, alkyl, alkenyl, alkynyl, cycloalk(en)yl, cycloalk(en)yl-alk(en/yn)yl, or optionally substituted phenyl-alk(en/yn)yl; A is a bond or a hydrocarbon spacer group; B is a group -CR_a(NR_bR_c)-COOR₅ wherein R_a-R_c are independently hydrogen or alkyl, and R₅ is defined as R₁ or pivaloyloxymethyl, or B is a group of Formula (III), wherein R₂, R₃ and R₄ are independently selected from hydrogen, a non-aromatic hydrocarbon group, phenyl- and thienyl-alkyl, and a hetero aliphatic group, or R₃ and R₄ are connected, thereby forming an alkylene, alkenylene or alkynylene group, or R₄ and R₂ are connected in order to form an alkylene, alkenylene or alkynylene group, optionally substituted with hydroxy or methyl, or to form CH₂-O-CH₂; E is COOR₆, where R₆ is defined as R₅, or E is a tetrazolyl or triazolyl; X is O or S; and Y is O or S; are excitatory amino acid (EAA), in particular AMPA and/or NMDA receptor ligands, useful in the treatment of cerebral ischaemia, Huntington's disease, epileptic disorders, Parkinson's disease, Alzheimer's disease, schizophrenia, pain, depression and anxiety.</p>				

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3-Alkoxyisoxazol-4-yl-substituted 2-Amino Carboxylic Acid Compounds

Field of the Invention.

- 5 The present invention relates to a novel class of (3-alkoxyisoxazol-4-yl)-substituted 2-amino carboxylic acid derivatives and sulfur analogues thereof. The compounds are excitatory amino acid (EAA) receptor ligands, in particular AMPA and/or NMDA receptor ligands useful in the treatment of cerebral ischaemia, Huntington's disease, epileptic disorders, Parkinson's disease, Alzheimer's disease, schizophrenia, pain, depression and anxiety.

10

Background of the Invention.

- As a result of extensive studies of excitatory mechanisms in the central nervous system (CNS) during the past three decades, there is now a consensus of opinion that (S)-glutamate
15 (Glu) is the major EAA neurotransmitter in the CNS (Lodge, D. *Excitatory Amino Acids in Health and Disease*. J. Wiley & Sons: Chichester, 1988; Wheal, H.; Thomson, A. *Excitatory Amino Acids and Synaptic Transmission*. Academic Press: London, 1991; Meldrum, B.S. *Excitatory Amino Acid Antagonists*. Blackwell Sci. Publ.: Oxford, 1991; Krogsgaard-Larsen, P.; Hansen, J.J. *Excitatory Amino Acid Receptors: Design of Agonist and Antagonists*. E.
20 Horwood: Chichester, 1992). Glu-operated neurotransmission is mediated by a large number of receptors, classified into at least four heterogeneous families of receptors named NMDA, AMPA, kainic acid, and metabotropic classes of receptors (Monaghan, D.T., et al. *Ann. Rev. Pharmacol. Toxicol.* 1989,29, 365-402; Watkins, J.C.; Krogsgaard-Larsen, P.; Honoré, T. *Trends Pharmacol. Sci.* 1990,11, 25-33; Simon, R.P. *Excitatory Amino Acids*. Thieme Med.
25 Publ.: New York, 1992).

- There is very strong evidence supporting the view that excessive excitation mediated by EAA receptors ("excitotoxicity") is a factor of major importance in cerebral ischaemia following stroke, head injury, asphyxia, subarachnoid haemorrhage, cardiac arrest and other situations
30 (Lodge, D., 1988 *supra*; Meldrum, B.S., 1991 *supra*). It has been shown in animal models that the damages caused by various ischaemic conditions can be inhibited by the administration of Glu-antagonists. So, although the relative importance of the different classes of EAA receptors in the phenomena underlying ischaemic insults is unclear, it is generally agreed that EAA receptor antagonists are potential therapeutic agents in these conditions.

35

Accumulating evidence derived from different lines of neurochemical and pharmacological research suggests that derailed EAA receptor mechanisms, possibly including "excitotoxicity", play a role in Huntington's disease (Young, A.B.; et al. *Science* 1988,241,

981-983), epileptic disorders (Krogsgaard-Larsen, P.; Hansen, J.J., 1992 *supra*), Parkinson's disease (Klockgether, T.; Turski, L. *Trends. Neurosci.* 1989,12, 285-286), and Alzheimer's disease (Greenamyre, J.T.; Maragos, W.F. *Cerebrovasc. Brain. Metab. Rev.* 1993,5, 61-94; Francis, P.T., et al. *J. Neurochem.* 1993,60, 1589-1604).

5

Furthermore, central EAA receptors may be involved in the synaptic mechanisms underlying schizophrenia (Reynolds, G.P. *Trends. Pharmacol. Sci.* 1992,13, 116-121), pain and anxiety (Drejer, J. In: *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists* (Eds. Krogsgaard-Larsen, P.; Hansen, J.J.) E. Horwood: Chichester 1992, pp. 352-375) and depression (Trullas, R., Skolnick, P., *Eur. J. Pharmacol.* 1990, 185, 1-10 and Trullas et al., *Eur. J. Pharmacol.* 1991, 203, 379-385. So, reduced function of EAA receptors (EAA hypoactivity) seems to play a role in, for example, schizophrenia (Deutsch, S.I.; et al. *Clin. Neuropharmacol.* 1989,12, 1-13) and some of the clinical symptoms seen in Alzheimer's disease (Greenamyre, J.T.; et al. *Prog. Neuro-Psychopharmacol. & Biol. Psychiat.* 1988,12, 421-430). It is possible that "excitotoxicity" as well as EAA hypoactivity are involved in the complex mechanisms associated with Alzheimer's disease (Greenamyre, J.T.; 1988 *supra*; Greenamyre, J.T.; Maragos, W.F., 1993, *supra*).

Accordingly, EEA receptor ligands are considered to be useful in the treatment of cerebral ischaemia, Huntington's disease, epileptic disorders, Parkinson's disease, Alzheimer's disease, anxiety, schizophrenia, depression and pain.

Most EAA receptor agonists so far tested, show more or less pronounced neurotoxicity in model systems and consequently clinical uses of such compounds may be limited (Carlsson, M.; Carlsson, A. *Trends. Neurosci.* 1990,13, 272-276) (Willetts, J.; Balster, R.L.; Leander, J.D. *Trends. Pharmacol. Sci.* 1990,11,423-428).

Partial EAA agonists showing appropriate balance between agonism and antagonism may, on the other hand, have considerable therapeutic interest, cf. the above indications, (Greenamyre, J.T.; 1988 *supra*; Christensen, I.T.; et al. *Drug. Des. Del.* 1989,5, 57-71; Francis, P.T.; et al. *J. Neurochem.* 1993,60, 1589-1604). Partial agonists may, by virtue of their EAA antagonist profile, show therapeutically useful neuroprotection and, at the same time, be sufficiently agonistic to prevent total blockade of the neurotransmission mediated by the particular EAA receptor.

35

ATPA, the 5-*tert*-butyl analogue of AMPA ((*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid), has been disclosed to be systemically active whereas it has not been reported to show neurotoxic effects in animals (Ornstein, P.L.; et al. *J. Med. Chem.* 1993,36,

2046-2048; Lauridsen, J.; Honoré, T.; Krogsgaard-Larsen, P. *J. Med. Chem.* 1985, 28, 668-672).

Like AMPA itself, a number of mono- and bicyclic AMPA analogues have been found to show selective agonist effects at AMPA receptors (Hansen, J.J.; Krogsgaard-Larsen, P. *Med. Res. Rev.* 1990, 10, 55-94; Krogsgaard-Larsen, P.; Hansen, J.J., 1992 *supra*). One of these analogues, (RS)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid (APPA), in which the methyl group of AMPA has been replaced by a phenyl group, shows a weak but unique partial agonist profile (Christensen, I.T.; et al., 1989, *supra*).

10

ACPA ((RS)-2-amino-3-(3-carboxyoxo-5-methylisoxazol-4-yl)propionic acid) has been described as a potent AMPA receptor agonist (Madsen, U. and Wong, E. *J. Med. Chem.* 1992, 35, 107-111).

15 Furthermore, WO-A1 95012587 discloses a class of (5-arylisoaxazol-4-yl)- or (5-arylisothiazol-4-yl)-substituted 2-amino carboxylic acid compounds as EAA-receptor ligands.

As seen from the above non-neurotoxic, CNS-active EEA receptor ligands with good penetration into the CNS are highly desirable for treating the various diseases mentioned and, accordingly, it is the object of the present invention to provide such new drugs.

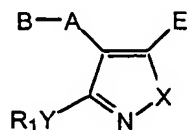
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Summary of the invention

It has now been found that a novel class of (3-alkoxyisoxazol-4-yl)-substituted 2-amino carboxylic acid derivatives and sulfur analogues thereof are EAA receptor ligands, in particular AMPA and/or NMDA receptor ligands.

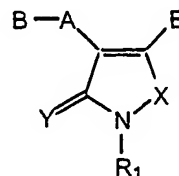
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Accordingly, the present invention relates to a novel class of compounds having general Formula I or II



I

or



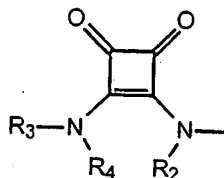
II

30

wherein R₁ is hydrogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, cycloalk(en)yl, cycloalk(en)yl-C₁₋₆ alk(en/yn)yl or phenyl-C₁₋₆ alk(en/yn)yl the phenyl group being optionally substituted with CF₃, halogen, C₁₋₆ alkyl or C₁₋₆ alkoxy;

A is a bond or a spacer group selected from C_{1-6} alkylene, C_{2-6} alkenylene or C_{2-6} alkynylene, and cycloalkylene;

B is a group $-CR_a(NR_bR_c)-COOR_5$ wherein $R_a - R_c$ are independently hydrogen or C_{1-6} alkyl, and R_5 is defined as R_1 or pivaloyloxymethyl, or B is a group of Formula III



III

wherein R_2 , R_3 and R_4 are independently selected from the group consisting of

- a) hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, cycloalk(en)yl, cycloalk(en)yl- C_{1-6} alk(en/yn)yl, phenyl- C_{1-6} alkyl, thienyl- C_{1-6} -alkyl, and
- 10 b) C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl in which one or more carbon atoms are replaced by N, O, and/or S; or

R_3 and R_4 are connected thereby forming a C_2-C_6 alkylene, C_2-C_6 alkenylene or C_2-C_6 alkynylene group; or

R_4 and R_2 are connected in order to form a C_1-C_3 alkylene, C_2-C_3 alkenylene or C_2-C_3 alkynylene group optionally mono- or di-substituted with hydroxy or methyl, or to form CH_2-O-CH_2 ;

E is $COOR_6$, where R_6 is defined as R_5 , or E is tetrazol-5-yl, 1,2,4-triazol-3-yl or 1,2,3-triazol-4-yl

X is O or S; Y is O or S; and

20 pharmaceutically acceptable salts thereof.

In another aspect, the invention relates to a method for the preparation of the novel compounds of Formula I or II.

25 In yet another aspect, the invention relates to a pharmaceutical composition comprising a novel compound of Formula I or II together with a suitable pharmaceutically acceptable carrier or diluent.

In yet another aspect, the invention relates to the use of a compound of Formula I or II for
30 preparing a pharmaceutical composition for treatment of cerebral ischaemia, Huntington's disease, epileptic disorders, Parkinson's disease, Alzheimer's disease, schizophrenia, pain, depression or anxiety.

Some compounds of the invention have been found to be AMPA receptor ligands with affinities in micromolar concentrations and some compounds have been found to bind to NMDA receptors. Furthermore, some of the compounds of the invention were found to be agonists whereas others were found to be antagonists. Thus the compounds of the invention are useful in the treatment of cerebral ischaemia, Huntington's disease, epileptic disorders, Parkinson's disease, Alzheimer's disease, schizophrenia, pain, depression and anxiety. The compounds wherein R_5 and/or R_6 are not hydrogen are prodrugs for the corresponding compounds wherein R_5 and R_6 are hydrogen.

10 Detailed description of the invention

Some of the compounds of general Formula I or II may exist as optical isomers thereof, and such optical isomers are also embraced by the invention.

- 15 In general Formula I and II, the term C_{1-6} alkyl is intended to mean a straight chain or branched alkyl group having from 1 to 6 C atoms, inclusive, such as methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, 2-methyl-2-propyl etc. Similarly, C_{2-6} alkenyl and C_{2-6} alkynyl designate such straight chain or branched groups having 2 to 6 C-atoms and C_{1-6} alkylene, C_{2-6} alkenylene and C_{2-6} alkynylene designate such branched or straight chain divalent groups.
- 20 Cycloalkyl designates such a group having 3-7 carbon atoms and the term C_{1-6} -alkoxy designates such groups having a C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl moiety as defined above

The term "alk(en/yn)yl" means that the group may be an alkyl, alkenyl or alkynyl group.

- 25 The term bond (defined for A) means that B may be attached directly to the 4-position of the isoxazole ring.

Halogen means fluoro, chloro, bromo or iodo.

- 30 Some of the compounds of the general Formula I or II may exist as pharmaceutically acceptable salts thereof which are also embraced by the invention.

The salts of the compounds of the general Formula I or II are salts formed with non-toxic organic acids, e.g. maleic, fumaric, benzoic, ascorbic, oxalic, tartaric, lactic and malic acid, or inorganic acids, e.g. hydrochloric, hydrobromic, sulfuric, phosphoric and nitric acid or they may be salts of inorganic bases such as alkali metal salts, e.g. sodium, potassium, or lithium salts, alkaline earth metal salts, e.g. calcium or magnesium salts, or ammonium salts or salts of organic bases.

In Formula I and II, A is preferably a bond or C₁-C₃ alkylene, most preferably methylene.

B is preferably -CR_a(NR_bR_c)-COOR₅ wherein R_b - R_c are hydrogen and R_a is hydrogen or C₁₋₆ alkyl, conveniently methyl, or a group of Formula III wherein R₂, R₃ and R₄ are hydrogen or C₁₋₆ alkyl, or R₄ and R₂ are connected in order to form a C₁-C₃ alkylene group. Most preferably, B is -CH(NH₂)-COOH or a group of Formula III wherein each of R₂, R₃ and R₄ are hydrogen.

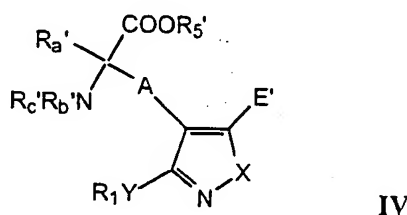
Preferably, E is COOH, triazolyl or tetrazolyl, preferably COOH. Another subgroup comprises the compounds wherein E is COOR₆ where R₆ is not H. According to a preferred subgroup of the compounds of the invention X and Y are O. Other subgroups are those wherein X is O and Y is S; Y is O and X is S; and X and Y are S, respectively.

R₁ is preferably C₁₋₆ alkyl, C₂₋₆ alkenyl or C₂₋₆ alkynyl. Particularly suitable R₁ groups are methyl, ethyl, propyl, butyl and propargyl.

In a preferred embodiment of the invention the compound is a compound of Formula I wherein A is a bond or C₁-C₃ alkylene, B is -CH(NH₂)-COOH or a group of Formula III wherein each of R₃, R₄ and R₂ are hydrogen, X and Y are both oxygen, and R₁ is C₁₋₆ alkyl, C₂₋₆ alkenyl or C₂₋₆ alkynyl. Particularly suitable R₁ groups are methyl, ethyl, propyl, butyl and propargyl.

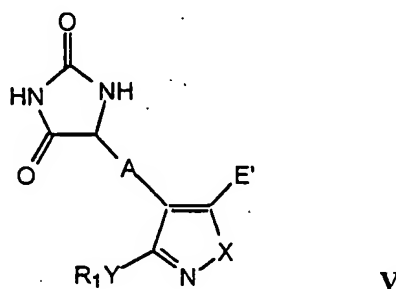
According to the invention, the compounds of Formula I or II are prepared by the following methods. For the sake of simplicity the reactions a) - e) and g) - h) are only shown for Formula I. Same methods may be used with respect to Formula II.

a) in order to obtain a compound of Formula I wherein B is a -CR_a(NR_bR_c)-COOR₅ wherein R_a - R_c and R₅ are as previously defined, and at least one of R_b, R₅ and R₆ is hydrogen, deprotection of a compound of the general Formula IV



wherein R₁, A, X and Y are as previously defined, R_a' - R_c', E' and R₅' are as defined for R_a - R_c, and E and R₅, respectively, or they are protection groups, provided that at least one of E', R₅' and R_c' is a protection group;

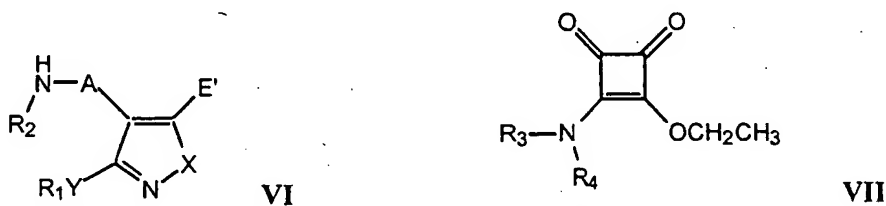
b) in order to obtain a compound of Formula I wherein B is a $-\text{CR}_b(\text{NR}_b\text{R}_c)-\text{COOR}_s$ group wherein R_b , R_c and R_s are all hydrogen, deprotection of a compound of the general Formula V



wherein R_1 , A, Y, X and E' are as previously defined;

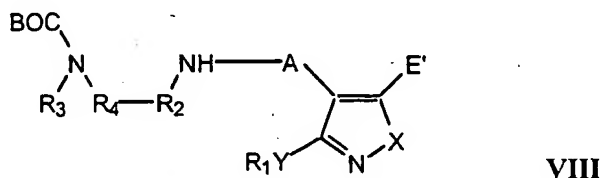
5

c) in order to obtain a compound of Formula I wherein B is a group of Formula III, addition-elimination reaction of a compound of the general Formula VI with a compound of the general Formula VII:



10 in which formulas R_1 - R_4 , A, X, Y and E' are previously defined;

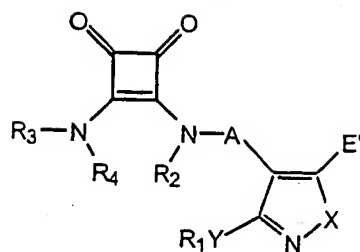
d) In order to obtain a compound of Formula I, wherein B is a group of Formula III wherein R^4 and R^2 are linked to form a C_{1-3} alkylene, $\text{C}_2\text{-C}_3$ alkenylene or $\text{C}_2\text{-C}_3$ alkynylene group optionally mono- or di-substituted with hydroxy or methyl, reacting a compound of Formula

15 **VIII**

wherein R_1 , R_3 , A, X, Y and E' are as previously defined; R_4 and R_2 are linked to form a group as defined above and BOC is t-butoxycarbonyl, with 3,4-diethoxy-3-cyclobuten-1,2-dione and subsequent ringclosure and deprotection;

20

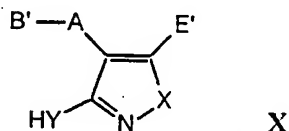
e) in order to obtain a compound of Formula I wherein B is a group of Formula III and one or more of R_2 - R_4 are different from hydrogen, alkylation of a compound of the general Formula IX



IX

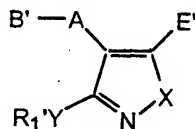
wherein R_1 , R_2 , R_3 , R_4 , A , X , Y and E' are as previously defined, at least one of $R_2 - R_4$, however, being hydrogen;

- 5 f) in order to obtain a compound of Formula I or II, alkylating a compound having general Formula X



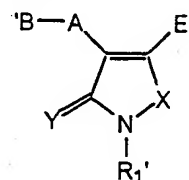
X

- wherein A , X , Y and E' are as previously defined and B' is as B except that in the definition of R_b , R_c and R_s hydrogen is replaced by a protection group,
10 with an alkylating agent $R_1'Z$ wherein R_1' is as R_1 except that it may not be hydrogen, thereby obtaining a mixture of the compounds XI and XII:



XI

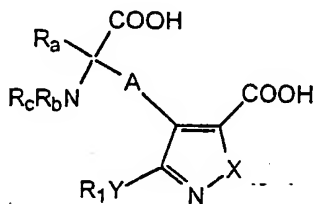
or



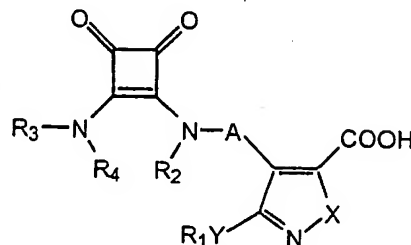
XII

- wherein A , X , Y , E' and B' are as defined above, and then separating and deprotecting the
15 compounds;

- g) in order to obtain a compound of Formula I wherein R_s and/or R_c is different from hydrogen, esterification of a compound of formula XIII or XIV:



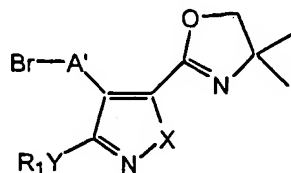
XIII



XIV

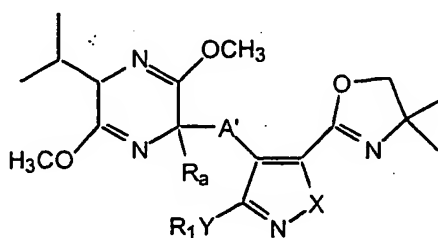
- 20 wherein R_1 , R_2 , R_3 , R_4 , A , X , Y and $R_a - R_c$ are as previously defined;

h) in order to obtain a compound of Formula I wherein B is a $-\text{CR}_s(\text{NR}_b\text{R}_c)-\text{COOR}_5$ group wherein R_s , R_b , R_c and R_5 are all hydrogen, and E is COOH in particular an enantiomeric pure compound, subjecting a compound of Formula XV



XV

- 5 to a Schöllkopf Bis-Lactim Amino Acid synthesis and subsequent deprotection of the obtained bislactim ether having Formula XVI



XVI

in which formulas X, Y, R_8 and R_1 are as previously defined and A' is as defined for A except that it may not be a bond.

10

In the method of the invention preferred protection groups are as follows:

For $\text{E}=\text{COOH}$: 4,5-dihydro-4,4-dimethyloxazol-2-yl, C_{1-6} alkyl or a benzyl group; for $\text{R}_5=\text{hydrogen}$: C_{1-6} alkyl and $\text{R}_6=\text{hydrogen}$: C_{1-6} alkylcarbonyl.

- 15 The one step deprotection according to method a) is carried out by treatment of the compound of Formula IV with a suitable aqueous acid, conveniently an 0.5-12 N aqueous solution of HCl, an aqueous solution of 48% HBr, or a saturated solution of HBr in acetic acid. The deprotection may also be carried out in successive steps by using aqueous acids and aqueous bases, conveniently successively in an aqueous acid such as 0.5-12 N HCl, an aqueous base such as 1-8 N NaOH and an aqueous acid such as 0.5-12 N HCl, or successively
- 20 in an aqueous base such as 1-8 N NaOH and an aqueous acid such as 0.5-12 N HCl.

- Starting materials of Formula IV are conveniently prepared from 3-alkoxy-4-methylisoxazole-5-carboxylic acid (WO95/12587, A1) by complete deprotection in an aqueous acid according to the above described deprotection conditions, optional esterification
- 25 of the 3-hydroxy-4-methylisoxazole-5-carboxylic acid and subsequent alkylation with an appropriate halide or simply by alkylation. This is followed by bromination of the 4-methylisoxazole group and subsequent alkylation with an amino acid precursor e.g. diethyl acetamidomalonate. Other 4-alkylisoxazoles may be prepared by chain-elongation, e.g. alkylation with cyanide or diethyl malonate and subsequent transformation to the primary

alkyl halide or aldehyde. The halide may be treated as outlined above. The aldehyde may be used as starting material for the preparation of compounds of general formula V.

In b), the one step deprotection is carried out by treatment of a compound of Formula V with a suitable aqueous acid or aqueous base, conveniently in 0.5-8 N aqueous hydrochloric acid.

- 5 The deprotection may also be performed in successive steps by using aqueous acids and aqueous bases as mentioned above for method a). The hydantoin ring may also be cleaved by the use of an aqueous solution of $\text{Ba}(\text{OH})_2$, aqueous 10-70% sulphuric acid or by the use of enzymes such as hydantoinases. The cleavage of the hydantoin ring may be carried out either before or after the deprotection of the E-group. The R_1 group may have to be reintroduced by alkylation after complete deprotection of the hydantoin intermediate.

10 The hydantoin rings in the compounds of the general Formula V are conveniently formed according to the methods described by Ware, E., *Chem. Rev.* 1950, 46, 403-470. The cleavage of the hydantoin ring is conveniently performed in analogy with the methods described by Curry, K. *et al J. Med. Chem.* 1988, 31, 864-867, Farrington, G.K. *et al, J. Med. Chem.* 1987, 30, 2062-2067, Grunewald, G.L. *et al, J. Med. Chem.* 1980, 23, 754-758, Hiroi, K. *et al, Chem. Pharm. Bull.* 1968, 16, 444-447 or Stark, G.R. *et al, J. Biol. Chem.* 1963, 238, 214-226.

15 The starting material for preparation of compounds of Formula V may be obtained as outlined above for starting materials for method a). If A is a bond, the aldehyde may be prepared from the bromomethyl compound by bromination and subsequent transformation into the aldehyde.

20 The addition-elimination reaction according to method c) is conveniently performed in a protic organic solvent such as an alcohol, preferably in the presence of a suitable inorganic base such as aqueous NaOH at room temperature. The intermediates of Formula VII may be prepared by the methods described by Cohen, S. *et al, J. Amer. Chem. Soc.* 1966, 88, 1533-1536, EP-A2-0496561 or Kinney, W.A. *et al, J. Med. Chem.* 1992, 35, 4720-4726.

The intermediate of the general formula VI is readily obtained by a Gabriel synthesis of primary amines as described by Sheehan, J. C. *et al., J. Am. Chem. Soc.*, 1950, 72, 2786-88. The alkyl halide starting materials for this synthesis are conveniently obtained as described with respect to starting materials used in method a), cf. above.

- 30 The deprotection is conveniently performed by the use of an aqueous acid or an aqueous base, preferably 0.5-8 N HCl or aqueous 0.5-8 N NaOH, either at room temperature or at elevated temperatures.

In method d), the reaction and the subsequent ringclosure and deprotection are performed as described by Kinney *et al.*, EP-A2-0496561.

- 35 The starting materials of formula VIII may be obtained by reacting e.g. 4-bromomethyl isoxazole obtained as described with respect to the starting materials in method a) with a mono-BOC-protected alkylene diamine cf. EP-A2-0496561.

The alkylation of compounds of the general Formula IX according to method e) is conveniently performed in an inert organic solvent such as a suitable alcohol, ketone or dimethylformamide preferably in the presence of a suitable base such as sodium hydride, potassium carbonate or triethylamine, as described by Kinney, W.A., EP-A2-0496561. The starting materials of formula IX may be obtained by method c).

In method f), deprotection of compounds of general Formulas XI and XII is accomplished as described in method a) or by using a solution of hydrochloric acid in diethyl ether or another non-aqueous deprotection method. Starting material X is obtained as described with respect to the starting materials in method a) above.

In method g), the esterification may be performed by methods well known in the art, e.g. treatment with an acidic solution of an alcohol. Starting materials are prepared in accordance with method a) - e) or h).

Resolution of the compounds of general formula I is conveniently performed by diastereomeric saltformation using optical active acids or bases, e.g. 1-phenylethylamine. In some cases, the resolution is conveniently performed by formation of diastereomeric compounds and subsequently separation of the diastereomers by flash chromatography or crystallisation. Certain diastereomers may conveniently be prepared by asymmetric synthesis by using Schöllkopf's Bis-Lactim Amino Acid synthesis, cf. method h). In this synthesis, starting materials are alkyl halides obtained as described above for starting materials for method a). The protecting group for the 5-carboxyisoxazole group is preferably an 2-oxazoline group prepared from the corresponding 5-cyanoisoxazole (WO95/12587, A1) by condensation with an aminoalcohol.

Salts of the compounds of the invention are easily prepared by methods well known in the art, i.e. by reacting the compound with either the equivalent amount of acid or base in an aqueous miscible solvent, such as acetone or ethanol, with isolation of the salt by concentration and cooling, or reacted with an excess of the acid or base in an aqueous immiscible solvent such as ethyl ether or chloroform, with the desired salt separating directly. These salts may also be prepared by the classical method of double decomposition of appropriate salts.

The compounds of general Formula I and the pharmaceutically acceptable acid addition salts thereof may be administered in any suitable way, e.g. orally or parenterally, and the compounds may be presented in any suitable form for such administration, e.g. in the form of tablets, capsules, powders, syrups or solutions or dispersions for injection.

An effective daily dose of a compound of general Formula I or a pharmaceutically acceptable salt thereof is from 10 µg/kg to 50 mg/kg body weight.

Examples

5

In the following the invention is further illustrated by way of examples which may in no way be construed as limiting for the invention.

All melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. ¹H
10 NMR and ¹³C NMR spectra were recorded on a Bruker 250 MHz spectrometer (250.13 MHz for ¹H NMR and 62.90 MHz for ¹³C NMR) using TMS as an internal standard if not otherwise stated.

Mass spectra were obtained on a Quattro MS-MS system from VG Biotech, Fisons
15 Instruments connected to an HP 1050 modular HPLC system. 20-50 µl of sample (10 µg/ml) dissolved in a mixture of 1% acetic acid in acetonitrile/water = 1:1 or in a mixture of acetonitrile/water/aqueous ammonia (25%) = 25:25:1 (zwitterions) was introduced via the autosampler at a flow of 30 µl/min into the Electrospray Source. Spectra were recorded at standard conditions to obtain molecular weight information ((M + H)⁺) or ((M - H)). The
20 background was subtracted.

Analytical HPLC was carried out on a 150 x 4.6 mm Lichrocart 250-4 (Merck) column eluted at 35°C with 1 mL/min of methanol/0.01 M ammonium acetate, pH 8 = 3:2. The instrumentation used consisted of a L6200 HPLC pump, a L5025 column thermostat and a L4000A
25 UV-VIS detector (set at 230 nm). Diastereomeric purities expressed as diastereomeric excess (de) were calculated from peak areas.

Chiral HPLC analysis was performed on a 150 x 4.6 mm Sumichiral OA-5000 column eluted at ambient temperature with 1 mL/min of 5 mM CuSO₄ (aq). The instrumentation used
30 consisted of an AS 2000 autosampler, a L6200 HPLC pump, a T6300 column thermostat, a L4250 UV-VIS detector (set at 240 nm), and a D 6000 computer interface, all from Merck-Hitachi. Enantiomeric purities expressed as enantiomeric excess (ee) were calculated from peak areas.

35

Example 1**(*RS*)-2-Amino-3-(5-carboxy-3-methoxyisoxazol-4-yl)propionic Acid Hydrate (Comp. 1)****1) 3-Hydroxy-4-methylisoxazole-5-carboxylic Acid**

3-Ethoxy-4-methylisoxazole-5-carboxylic acid (15 g, 88 mmol) and 47% HBr (aq) (150 mL) was boiled under reflux for 6 h. The solution was cooled and crystalline title compound was collected by filtration (8.7 g, 69%): mp 257-259 °C. The acidic filtrate was added water (100 mL), and extracted with diethyl ether (6 x 400 mL). The organic extracts were washed with brine (100 mL), dried (MgSO₄) and concentrated in vacuo to give crude title compound (3.0 g, 24%). Overall yield of 93%. A mixture of the two crops were used in the next step.

10

2) Ethyl 3-Hydroxy-4-methylisoxazole-5-carboxylate

3-Hydroxy-4-methylisoxazole-5-carboxylic acid (6.0 g, 42 mmol) and a saturated solution of HCl in EtOH (110 mL) was boiled under reflux for 4 h. The solution was concentrated in vacuo and the residue dissolved in EtOAc, dried (MgSO₄) and evaporated in vacuo to give crude title compound (7.2 g, 100%). A small sample was recrystallized (EtOAc/heptane) to give colorless crystals: mp 133-134 °C. The crude product was used in the next step without further purification.

3) Ethyl 3-Methoxy-4-methylisoxazole-5-carboxylate

A mixture of ethyl 3-hydroxy-4-methylisoxazole-5-carboxylate (1.0 g, 5.8 mmol), methyl iodide (0.4 mL, 5.8 mmol) and K₂CO₃ (1.6 g, 11.7 mmol) in DMF (40 mL) was heated at 40 °C for 1 h. The mixture was poured onto an ice/water mixture (100 mL) and extracted with diethyl ether (3 x 100 mL). The organic extracts were washed with water (2 x 50 mL), brine (50 mL), dried (MgSO₄) and concentrated in vacuo (0.8 g, 74%). The procedure was repeated in order to obtain crude product equivalent to 17.5 mmol of starting material which was subjected to flash chromatography (silica gel, eluent: dichloromethane/diethyl ether = 9:1) affording crude title compound as a yellow oil (1.4 g, 43%) which was used in the next step without further purification.

4) Ethyl 4-(Bromomethyl)-3-methoxyisoxazole-5-carboxylate

Ethyl 3-Methoxy-4-methylisoxazole-5-carboxylate (1.3 g, 7.0 mmol), NBS (1.4 g, 7.9 mmol), dibenzoyl peroxide (catalytic amount) and tetrachloromethane (40 mL) was boiled under reflux for 10 h. The mixture was cooled, filtered and concentrated in vacuo to give crude title compound as a yellow oil (1.8 g, 97%). The crude product was used in the next step without further purification.

5) *Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[5-(ethoxycarbonyl)-3-methoxyisoxazol-4-yl]propionate*

A mixture of diethyl acetamidomalonate (1.6 g, 7.4 mmol) and potassium *tert*-butoxide (0.9 g, 8.0 mmol) in *N*-methylpyrrolidone (30 mL) was stirred at room temperature for 30 min. Ethyl 4-(bromomethyl)-3-methoxyisoxazole-5-carboxylate (1.8 g, 6.8 mmol) in *N*-methylpyrrolidone (10 mL) was added (temp 22-28 °C) and the resulting mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured onto an ice/water mixture (100 mL) and the aqueous phase was extracted with EtOAc (3 x 150 mL). The organic extracts were washed with an aqueous solution of potassium *tert*-butoxide, water (100 mL) and brine (100 mL), dried (MgSO₄) and concentrated in vacuo. Flash chromatography (silica gel, eluent: EtOAc/heptane = 1:1) afforded crude title compound (1.8 g, 66%). A small sample was recrystallized (EtOAc/heptane) to give colourless crystals: mp 78-80 °C. The crude product was used in the next step without further purification.

6) *(RS)-2-Amino-3-(5-carboxy-3-methoxyisoxazol-4-yl)propionic Acid Hydrate (Comp. 1)*

A suspension of ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[5-(ethoxycarbonyl)-3-methoxyisoxazol-4-yl]propionate (1.2 g, 3.0 mmol) in 0.5 M HCl (100 mL) was boiled under reflux for 48 h. The mixture was cooled, washed with dichloromethane (100 mL) and diethyl ether (2 x 100 mL), filtered and concentrated in vacuo. Water was added (5 mL) and the pH adjusted to about 3 by addition of NaOH (0.1 M and 1 M). The aqueous phase was reduced in vacuo (2 mL) and a precipitate collected by filtration. The precipitate was stirred in water (2 mL) at room temperature for 24 h affording Comp. 1 after filtration (70 mg, 10%): mp 222-225 °C (dec); ¹H NMR (DMSO-*d*₆) δ 2.88 (dd, 1 H), 3.01 (dd, 1 H), 3.85-3.96 (m, 1 H), 3.90 (s, 3 H); ¹³C NMR (DMSO-*d*₆) δ 22.70, 52.38, 57.32, 103.25, 159.43, 165.95, 170.66 (2 C); MS ((M + H)⁺) *m/z* 231. Anal. (C₈H₁₀N₂O₆ · 0.25H₂O) calcd, C 40.94, H 4.51, N 11.94; found, C 41.01, H 4.37, N 11.91.

The following compounds were prepared in a similar way:

(RS)-2-Amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic Acid (Comp. 2).

Mp 238-240 °C (dec); ¹H NMR (DMSO-*d*₆) δ 1.34 (t, 3 H), 2.90 (dd, 1 H), 3.03 (dd, 1 H), 3.96 (dd, 1 H), 4.23 (q, 2 H); ¹³C NMR (DMSO-*d*₆) δ 14.46, 22.41, 51.89, 65.63, 103.34, 159.22, 164.97, 169.75, 170.40; MS ((M + H)⁺) *m/z* 245. Anal. (C₉H₁₂N₂O₆) calcd, C 44.27, H 4.95, N 11.47; found, C 44.10; H 4.92, N 11.34.

(RS)-2-Amino-3-(5-carboxy-3-isopropoxyisoxazol-4-yl)propionic Acid (Comp. 3).

Mp 242-243 °C (dec); ¹H NMR (DMSO-*d*₆) δ 1.32 (dd, 6 H), 2.88 (dd, 1 H), 3.01 (dd, 1 H), 3.96 (dd, 1 H), 4.79 (h, 1 H); ¹³C NMR (DMSO-*d*₆) δ 21.57, 21.77, 22.35, 51.82, 73.13,

103.56, 159.22, 164.91, 169.08, 170.36; MS ((M + H)⁺) *m/z* 259. Anal. (C₁₀H₁₄N₂O₆) calcd, C 46.51, H 5.46, N 10.85; found, C 46.37, H 5.46, N 10.83.

(*RS*)-2-Amino-3-(5-carboxy-3-hydroxyisoxazol-4-yl)propionic Acid Hydrate (Comp. 4)

5 Mp 175-177 °C; ¹H NMR (DMSO-*d*₆) δ 3.00 (d, 2 H), 3.88 (t, 1 H); ¹³C NMR (DMSO-*d*₆) δ 23.07, 52.07, 105.84, 159.41, 162.11, 169.89, 170.78; MS ((M + H)⁺) *m/z* 217. Anal. (C₇H₈N₂O₆ · 0.25H₂O) calcd, C 38.10, H 3.88, N 12.70; found, C 37.72, H 3.98, N 12.52.

Example 2

10 (*RS*)-2-Amino-3-(5-carboxy-2,3-dihydro-2-methyl-3-oxoisoxazol-4-yl)propionic Acid Hydrate (Comp 5)

Ethyl 2,3-Dihydro-2,4-dimethyl-3-oxoisoxazole-5-carboxylate

A mixture of ethyl 3-hydroxy-4-methylisoxazole-5-carboxylate (2.0 g, 11.7 mmol) and K₂CO₃ (4.0 g, 29 mmol) in ethanol (50 mL) was heated at 40 °C for a total of 26 h. Methyl iodide (0.8 mL, 13 mmol) was added after 1 h and an additional 3 times during the next 25 h. The solution was filtered and reduced in vacuo (according to ¹H NMR, a 1:1 mixture of the title compound and ethyl 3-methoxy-4-methylisoxazole-5-carboxylate was obtained). Flash chromatography (silica gel, eluent: dichloromethane/diethyl ether = 9:1 then 1:1) gave ethyl 3-Methoxy-4-methylisoxazole-5-carboxylate as a yellow oil (0.40, 18%) and title compound 20 (0.45 g, 21%). A small sample of the latter was recrystallized (EtOAc/heptane) to give colorless crystals: mp 64-65 °C. Crude title compound was used in the next step without further purification.

25 (*RS*)-2-Amino-3-(5-carboxy-2,3-dihydro-2-methyl-3-oxoisoxazol-4-yl)propionic Acid Hydrate (Comp 5)

The title compound was obtained by processes analogous to those of steps 2) - 6) of Example 1 using the product of 1) above (70 mg, colourless crystals, 72%). Mp 211-212 °C (dec); ¹H NMR (DMSO-*d*₆) δ 2.87 (dd, 1 H), 2.97 (dd, 1 H), 3.43 (s, 3 H), 3.92 (dd, 1 H); ¹³C NMR (DMSO-*d*₆) δ 23.10, 32.32, 51.79, 106.51, 158.59, 162.37, 166.64, 170.35; MS ((M + 30 H)⁺) *m/z* 231. Anal. (C₈H₁₀N₂O₆ · 0.25H₂O) calcd, C 40.94, H 4.51, N 11.94; found, C 40.93, H 4.55, N 11.71.

The following compound was prepared in a similar way:

35 (*RS*)-2-Amino-3-(5-carboxy-2-ethyl-2,3-dihydro-3-oxo-isoxazol-4-yl)propionic Acid Mono-hydrate (Comp. 6).

¹H NMR (D₂O, 1,4-dioxane d 3.70) δ 1.28 (t, 3 H), 3.19 (d, 2 H), 4.01 (q, 2 H), 4.18 (t, 1 H); ¹³C NMR (D₂O, 1,4-dioxane δ 67.40) δ 12.87, 23.85, 42.31, 53.27, 110.57, 159.88, 162.65,

166.67, 172.55; MS ((M + H)⁺) *m/z* 245. Anal. (C₉H₁₂N₂O₆ · H₂O) calcd, C 41.22, H 5.38, N 10.68; found, C 41.28, H 4.74, N 10.27.

Example 3

- 5 (S)-2-Amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic Acid (Comp (S)-2)
(R)-2-Amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic Acid (Comp (R)-2)

1) 5-(4,5-Dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxy-4-methylisoxazole
3-Ethoxy-4-methylisoxazole-5-carbonitrile (2.6 g, 17.1 mmol), 5.4 M NaOMe in MeOH (0.6
10 mL, 3.4 mmol) and EtOH (80 mL) was stirred at room temperature for 30 min. Acetic acid
(2.2 mL, 39.3 mmol) and 2-amino-2-methylpropan-1-ol (1.8 mL, 18.8 mmol) were added,
and the resulting mixture was boiled under reflux for 20 h. The reaction mixture was cooled,
added water (100 mL) and extracted with EtOAc (3 x 100 mL). The organic extracts were
washed with 1 M NaOH (50 mL), brine, dried (MgSO₄) and evaporated in vacuo. The residue
15 was dissolved in EtOH (60 mL), a solution of KOH (1.8 g, 32 mmol) in water (12 mL) was
added, and the mixture was stirred at room temperature for 20 h. EtOH was removed in
vacuo, water was added (80 mL) and the aqueous phase extracted with EtOAc (3 x 100 mL).
The organic extracts were washed with brine, dried (MgSO₄) and evaporated in vacuo. Flash
chromatography (silica gel, eluent: EtOAc/heptane/triethylamine = 75:25:1) gave crude title
20 compound as a yellow oil (2.0 g, 52%)

2) 4-(Bromomethyl)-5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazole
5-(4,5-Dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxy-4-methylisoxazole (2.0 g, 8.9 mmol),
NBS (1.75 g, 9.8 mmol) and tetrachloromethane (150 mL) was boiled under reflux for 5 h.
25 The mixture was cooled, filtered and concentrated in vacuo. Flash chromatography (silica gel,
eluent: toluene/EtOAc/triethylamine = 100:10:1) gave the title compound as a yellow oil (2.0
g, 74%).

3) (2S,5R)-2,5-Dihydro-2-[[5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazol-4-
30 yl]methyl]-5-isopropyl-3,6-dimethoxypyrazine and (2R,2R)-2,5-Dihydro-2-[[5-(4,5-dihydro-
4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazol-4-yl]methyl]-5-isopropyl-3,6-dimethoxy-
pyrazine

A 1.6 M solution of butyllithium in hexane (1.9 mL, 3.0 mmol) was added to a precooled (-
78 °C) solution of (2R)-(-)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine (0.5 mL, 2.8
35 mmol) in anhydrous tetrahydrofuran (8 mL). Stirring was continued at -78 °C for 10 min, 4-
(bromomethyl)-5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazole (0.85 g, 2.8
mmol) dissolved in tetrahydrofuran (5 mL) was added and the resulting mixture stirred at -78
°C for 4.5 h. The reaction mixture was allowed to warm to room temperature and

concentrated in vacuo. The residue was dissolved in diethyl ether (40 mL) and poured onto an ice/water mixture (40 mL). The layers were separated and the aqueous phase extracted with diethyl ether (2 x 40 mL). The organic extracts were washed with brine, dried (MgSO₄) and concentrated in vacuo. Flash chromatography (silica gel, eluent: heptane/EtOAc = 3:1) gave the (2*S*,5*R*)-title compound as a yellow oil (0.65 g, 57%): de = 99.2% (retention time ca. 38 min). Further elution afforded crude (2*R*,5*R*)-title compound as a yellow oil (38 mg, 3%)

4) (2*R*,5*S*)-2,5-Dihydro-2-[[5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazol-4-yl]methyl]-5-isopropyl-3,6-dimethoxypyrazine and (2*S*,5*S*)-2,5-Dihydro-2-[[5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazol-4-yl]methyl]-5-isopropyl-3,6-dimethoxypyrazine

The title compounds were obtained by a procedure as described in step 3) above using (2*S*)-(+)-2,5-dihydro-2-isopropyl-3,6-dimethoxypyrazine as starting material. Flash chromatography (silica gel, eluent: heptane/EtOAc = 3:1) to give (2*R*,5*S*)-title compound as a yellow oil (0.8 g, 54%): de >99.2% (retention time ca. 38 min). Further elution afforded crude (2*S*,5*S*)-title compound as a yellow oil (60 mg, 4%).

5) (*S*)-2-Amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic Acid (Comp (*S*)-2)

A suspension of (2*S*,5*R*)-2,5-dihydro-2-[[5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazol-4-yl]methyl]-5-isopropyl-3,6-dimethoxypyrazine (0.6 g, 1.5 mmol) in 1 M trifluoroacetic acid (200 mL) was boiled under reflux for 5 h. The reaction mixture was concentrated in vacuo (2 mL), the residue dissolved in water (50 mL) and washed with EtOAc (3 x 50 mL). The aqueous phase was filtered, evaporated in vacuo to dryness and the residue treated with water (10 mL). The precipitate which formed was stirred at room temperature for 24 h, collected by filtration and recrystallized (water) to afford compound (*S*)-2 as colourless crystals (0.12 g, 33%): mp 259-261 °C (dec); ee >99% (retention time ca. 30 min); ¹H NMR (DMSO-*d*₆) δ 1.34 (t, 3 H), 2.90 (dd, 1 H), 3.03 (dd, 1 H), 3.96 (dd, 1 H), 4.23 (q, 2 H); MS ((M + H)⁺) *m/z* 245. Anal. (C₉H₁₂N₂O₆) calcd, C 44.27, H 4.95, N 11.47; found, C 44.45, H 4.96, N 11.46.

6) (*R*)-2-Amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic Acid (Comp. (*R*)-2)

A stirred solution of (2*R*,5*S*)-2,5-dihydro-2-[[5-(4,5-dihydro-4,4-dimethyl-1,3-oxazol-2-yl)-3-ethoxyisoxazol-4-yl]methyl]-5-isopropyl-3,6-dimethoxypyrazine (0.6 g, 1.5 mmol) and MeOH (7 mL) was added 0.25 M HCl (74 mL, 7.4 mmol), and the resulting mixture stirred at room temperature for 2 h. pH was adjusted to about 7 by addition of aqueous ammonia (0.5 M) and the MeOH removed in vacuo. pH was adjusted to 8-9 by addition of aqueous ammonia (0.5 M) and the aqueous phase extracted with EtOAc (4 x 50 mL). The organic extracts were washed with brine, dried (MgSO₄) and concentrated in vacuo. The residue was

suspended in 1 M HCl and the mixture boiled under reflux for 4.5 h. The reaction mixture was concentrated in vacuo (2 mL), the residue dissolved in water (50 mL) and washed with EtOAc (3 x 50 mL). The aqueous phase was filtered, evaporated in vacuo to dryness and the residue treated with water (10 mL). The precipitate which formed was stirred at room temperature for 2 h, collected by filtration and recrystallized (water) to afford compound (R)-2 as colorless crystals (0.13 g, 36%): mp 258-260 °C (dec); ee >99% (retention time ca. 50 min); ¹H NMR (DMSO-*d*₆) δ 1.34 (t, 3 H), 2.90 (dd, 1 H), 3.03 (dd, 1 H), 3.96 (dd, 1 H), 4.23 (q, 2 H); MS ((M + H)⁺) *m/z* 245. Anal. (C₉H₁₂N₂O₆) calcd, C 44.27, H 4.95, N 11.47; found, C 44.56, H 4.95, N 11.53.

10

Example 4

(*RS*)-2-Amino-3-[3-ethoxy-5-(1*H*-1,2,4-triazol-3-yl)isoxazol-4-yl]propionic Acid Hydrate (Comp 7)

N-[(*Dimethylamino*)methylidene]-3-ethoxy-4-methylisoxazole-5-carboxamide

15 A solution of 3-ethoxy-4-methylisoxazole-5-carboxamide (3.5 g, 21 mmol) in *N,N*-dimethylformamide dimethyl acetal (15 mL) was stirred at 120 °C for 15 min. After being cooled, the title compound was collected as a colourless crystals (4.2 g, 91%).

3-(3-Ethoxy-4-methylisoxazol-5-yl)-1*H*-1,2,4-triazole

20 To a solution of hydrazine hydrate (0.6 mL, 12.4 mmol) in acetic acid (15 mL) was added *N*-(dimethylamino)methylidene 3-ethoxy-4-methylisoxazole-5-carboxamide (1.8 g, 8.0 mmol). The reaction mixture was stirred at 90 °C for 15 min and then left at room temperature to crystallize affording pure title compound (1.2 g, 77%): mp 194-196 °C. Water was added (40 mL) and the aqueous phase was extracted with EtOAc (3 x 30 mL). The organic extracts were washed with brine, dried (MgSO₄) and concentrated in vacuo to give crude title compound 25 (0.3 g, 20%). The two crops were combined.

3-(3-Ethoxy-4-methylisoxazol-5-yl)-1-trityl-1*H*-1,2,4-triazole

30 *3*-(3-Ethoxy-4-methylisoxazol-5-yl)-1*H*-1,2,4-triazole (1.1 g, 5.7 mmol), triethylamine (2.5 mL, 18 mmol) and DMF (20 mL) was added trityl chloride (1.6 g, 5.7 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 5 h and poured onto an ice/water mixture (200 mL). The aqueous phase was extracted with diethyl ether (3 x 200 mL) and the organic extracts were washed with an aqueous solution of Na₂CO₃ (10%) (200 mL) and brine (200 mL). The solution was dried (Na₂SO₄) and concentrated in vacuo to give crude title 35 compound (2.5 g). A small sample was crystallized (EtOAc) to give a single isomer as colourless crystals: mp 181-183 °C. The crude product was used in the next step without further purification.

3-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-1-trityl-1H-1,2,4-triazole

A mixture of 3-(3-ethoxy-4-methylisoxazol-5-yl)-1-trityl-1H-1,2,4-triazole (2.4 g, 5.5 mmol) and NBS (1.1 g, 6.2 mmol) in tetrachloromethane (150 mL) was boiled under reflux for 3 h.

- 5 The reaction mixture was cooled, filtered and concentrated in vacuo to give crude title compound (2.8 g). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-3-[3-ethoxy-5-(1-trityl-1H-1,2,4-triazol-3-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate

- 10 A mixture of diethyl acetamidomalonate (1.3 g, 6.0 mmol) and potassium *tert*-butoxide (0.73 g, 6.5 mmol) in *N*-methylpyrrolidone (30 mL) was stirred at room temperature for 30 min. 3-[4-(Bromomethyl)-3-ethoxyisoxazol-5-yl]-1-trityl-1H-1,2,4-triazole (2.8 g, 5.4 mmol) in *N*-methylpyrrolidone (20 mL) was added (temp 22-28 °C) and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was poured onto an ice/water mixture (250 mL) and the aqueous phase was extracted with EtOAc (3 x 250 mL). The organic extracts were washed with an aqueous solution of potassium *tert*-butoxide and brine, dried (Na₂SO₄) and concentrated in vacuo. Flash chromatography (silica gel, eluent: EtOAc/heptane/triethylamine = 50:50:2) gave the title compound (2.2 g, 62%): mp 145-149 °C.

- 20 *(RS)*-2-Amino-3-[3-ethoxy-5-(1H-1,2,4-triazol-3-yl)isoxazol-4-yl]propionic Acid Hydrate (Comp 7)

- A suspension of ethyl 2-acetamido-3-[3-ethoxy-5-(1-trityl-1H-1,2,4-triazol-3-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate (1.5 g, 2.3 mmol) in 1 M HCl (150 mL) was boiled under reflux for 24 h. The solution was cooled, washed with diethyl ether (2 x 150 mL) and dichloromethane (150 mL), filtered and concentrated in vacuo. Water was added (5 mL) and the pH adjusted to about 3.5 by addition of NaOH (0.1 M and 1 M) affording Compound 7 by filtration (0.35 g, 56%): mp 225-227 °C (dec); ¹H NMR (DMSO-*d*₆) δ 1.38 (t, 3 H), 2.94 (dd, 1 H), 3.18 (dd, 1 H), 3.58 (dd, 1 H), 4.30 (q, 2 H), 8.64 (s, 1 H); ¹³C NMR (DMSO-*d*₆) δ 14.52, 23.60, 53.30, 65.93, 104.25, 146.18, 150.87, 158.44, 169.47, 170.51; MS ((M + H)⁺) *m/z* 268. Anal. (C₁₀H₁₃N₅O₄ · 0.25H₂O) calcd, C 44.20, H 5.01, N 25.77; found, C 44.42, H 5.29, N 25.52.
- 30

Example 5*(RS)*-2-Amino-3-[3-ethoxy-5-(5-tetrazolyl)isoxazol-4-yl]propionic acid (Comp. 8)

- 35 Was prepared by a method analogous to the method of Example 4 from ethyl 2-acetamido-3-[3-ethoxy-5-(tetrazol-5-yl)isoxazol-4-yl]-2-(ethoxycarbonyl)propionate.

Example 6**(*RS*)-2-Amino-3-(3-benzyloxy-5-carboxyisoxazol-4-yl)propionic Acid (Comp. 9)**

(*RS*)-2-Amino-3-(5-carboxy-3-hydroxyisoxazol-4-yl)propionic acid (3.5 g, 11.8 mmol) and a solution of HCl in ethanol (50 mL) was boiled under reflux for 2.5 h and evaporated to dryness in vacuo to give ethyl (*RS*)-2-amino-3-(5-ethoxycarbonyl-3-hydroxyisoxazol-4-yl)propionate (4.15 g, 100%).

A mixture of di-*tert*-butyl dicarbonate (3.1 g, 14 mmol), triethylamine (3.8 g, 37 mmol) and 1,4-dioxane (15 mL) was added to a solution of ethyl (*RS*)-2-amino-3-(5-ethoxycarbonyl-3-hydroxyisoxazol-4-yl)propionate (4.15 g, 11.7 mmol) in a water/1,4-dioxane (1:1) (50 mL), and the resulting mixture was stirred at room temperature for 16 h. The 1,4-dioxane was evaporated in vacuo, and the aqueous phase was acidified with dilute aqueous HCl. The aqueous phase was extracted with ethyl acetate, and the organic extracts washed with water, brine, dried (MgSO₄) and concentrated in vacuo. Flash chromatography (SiO₂, eluent: heptane/ethyl acetate/acetic acid (1:1, 4%)) gave ethyl (*RS*)-2-*tert*-butoxycarbonylamino-3-(5-ethoxycarbonyl-3-hydroxyisoxazol-4-yl)propionate as an oil (4.1 g, 92%).

A mixture of ethyl (*RS*)-2-*tert*-butoxycarbonylamino-3-(5-ethoxycarbonyl-3-hydroxyisoxazol-4-yl)propionate (3.2 g, 8.6 mmol), K₂CO₃ (2.4 g, 17.2 mmol) in acetone (40 mL) was heated to reflux temperature. Benzyl bromide (2.2 g, 12.9 mmol) was added, and the mixture was boiled under reflux for 1.5 h. Concentrated in vacuo and subjected to flash chromatography (SiO₂, eluent: heptane/ethyl acetate (2:1)) to give ethyl (*RS*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-benzyloxy-5-(ethoxycarbonyl)isoxazol-4-yl]propionate (1.64 g, 41%) and ethyl (*RS*)-2-[(*tert*-butoxycarbonyl)amino]-3-(2-benzyl-5-ethoxycarbonyl-2,3-dihydro-3-oxoisoxazol-4-yl)propionate (0.7 g, 18%).

A mixture of ethyl (*RS*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-benzyloxy-5-(ethoxycarbonyl)isoxazol-4-yl]propionate (0.65 mg, 1.4 mmol) and 1 M NaOH (50 mL) was boiled under reflux for 16 h. The mixture was cooled (5 °C), acidified with dilute aqueous HCl, and concentrated in vacuo. The residue was recrystallized from water to give (*RS*)-2-amino-3-(3-benzyloxy-5-carboxyisoxazol-4-yl)propionic acid (0.1 g, 23%): mp 209-211°C (dec); ¹H NMR (DMSO-*d*₆) δ 2.95 (dd, 1 H), 3.05 (dd, 1 H), 3.99 (t, 1 H), 5.26 (s, 2 H), 7.31-7.52 (m, 5 H); MS ((M + H)⁺) *m/z* 307. Anal. calcd, C 54.89, H 4.62, N 9.15; found, C 54.31, H 4.56, N 8.97.

The following compounds were prepared in a similar manner:

(*RS*)-2-Amino-3-(3-propoxy-5-carboxyisoxazol-4-yl)propionic Acid (Comp. 10)

Mp. 250-251 °C (dec). ¹H NMR (D₂O, dioxane, 1 M NaOD) δ 0.95 (t, 3 H), 1.76 (se, 2 H), 2.78 (dd, 1 H), 2.90 (dd, 1 H), 3.42 (dd, 1 H), 4.17 (t, 2 H). ¹³C NMR δ 12.3, 24.4, 29.8, 58.3,

74.9, 111.5, 164.3, 166.6, 173.9, 184.8. MS ((M+H)⁺) *m/z* 259. Anal. calcd, C 46.51, H 5.46, N 10.85; found C 46.43, H 5.41, N 10.54.

(RS)-2-Amino-3-(3-butoxy-5-carboxyisoxazol-4-yl)propionic Acid (Comp. 11)

5 Mp 238-240 °C (dec). ¹H NMR (D₂O, dioxane, 1 M NaOD) δ 0.95 (t, 3 H), 1.43 (se, 2 H), 1.76 (qui, 2 H), 2.8 (dd, 1 H), 2.91 (dd, 1 H), 3.44 (dd, 1 H), 4.25 (t, 2 H). ¹³C NMR NMR (D₂O, dioxan, 1 M NaOD) δ 13.79, 19.30, 27.90, 31.03, 56.39, 71.27, 109.65, 162.39, 164.72, 172.02, 182.92. MS ((M+H)⁺) *m/z* 273. Anal. calcd. C 48.53, H 5.92, N 10.29; found, C 48.80, H 5.99, N 10.34.

10

(RS)-2-Amino-3-(3-allyloxy-5-carboxyisoxazol-4-yl)propionic Acid (Comp. 12)

Mp 239-240 °C (dec). ¹H NMR (DMSO-*d*₆) δ 2.93 (dd, 1 H), 3.06 (dd, 1 H), 3.99 (dd, 1 H), 4.73 (d, 2 H), 5.29 (dd, 1 H), 5.44 (dd, 1 H), 6.05 (dq, 1 H).

15 Furthermore the following compounds are prepared similarly:

(RS)-2-Amino-3-[3-(*trans*-2-but-ene-oxy)-5-carboxyisoxazol-4-yl]propionic Acid

(RS)-2-Amino-3-[3-(3-methyl-2-but-ene-oxy)-5-carboxyisoxazol-4-yl]propionic Acid

20

Example 7

***(RS)*-2-Amino-3-(2-benzyl-5-carboxy-2,3-dihydro-3-oxoisoxazol-4-yl)propionic Acid, Hydrochloride, Monohydrate (Comp. 13)**

A mixture of ethyl *(RS)*-2-[(*tert*-butoxycarbonyl)amino]-3-(2-benzyl-5-ethoxycarbonyl-2,3-dihydro-3-oxoisoxazol-4-yl)propionate (0.9 g, 1.9 mmol) and 1 M HCl was boiled under reflux for 5 h. The mixture was evaporated in vacuo to dryness (0.56 g, 80%): mp 146-148°C (dec); ¹H NMR (DMSO-*d*₆) δ 3.08 (dd, 1 H), 3.19 (dd, 1 H), 4.17 (br s, 1 H), 5.16 (s, 2 H), 7.24-7.45 (m, 5 H); MS ((M + H)⁺) *m/z* 307. Anal. calcd, C 46.60, H 4.76, N 7.77; found, C 46.88, H 4.81, N 7.96.

30

Example 8

Benzyl *(RS)*-2-Amino-3-(5-benzyloxycarbonyl-3-ethoxyisoxazol-4-yl)propionate Hydrochloride (Comp. 14)

A mixture of di-*tert*-butyl dicarbonate (1.1 g, 4.9 mmol), NaHCO₃ (1.1 g, 13 mmol) and 1,4-dioxane (3 mL) was added to a solution of *(RS)*-2-amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic acid (1.0 g, 4.1 mmol) in a water/1,4-dioxane (1:1) (10 mL), and the resulting mixture was stirred at room temperature for 16 h. The 1,4-dioxane was evaporated in vacuo, and the aqueous phase was acidified with dilute aqueous HCl. The aqueous phase was

extracted with ethyl acetate, and the organic extracts washed with water and brine. Dried (MgSO_4), concentrated in vacuo and subjected to flash chromatography (SiO_2 , eluent: ethyl acetate/ethanol/acetic acid (3:1, 4%)) to give (*RS*)-2-[(*tert*-butoxycarbonyl)amino]-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic acid (1.4 g, 100%)

- 5 A mixture of (*RS*)-2-[(*tert*-butoxycarbonyl)amino]-3-(5-carboxy-3-ethoxyisoxazol-4-yl)-propionic acid (1.4 g, 4.1 mmol), benzyl bromide (1.4 g, 8.2 mmol) in benzene/ tetrahydrofuran (4:1) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (1.3 g, 8.6 mmol) and the resulting mixture was boiled under reflux for 3 h. The mixture was filtered and evaporated in vacuo. Flash chromatography (SiO_2 , eluent: ethyl acetate/heptane (1:3)) gave benzyl (*RS*)-2-
10 [(*tert*-butoxycarbonyl)amino]-3-(5-benzyloxycarbonyl-3-ethoxyisoxazol-4-yl)propionate as an oil (1.9 g, 86%).

- A mixture of benzyl (*RS*)-2-[(*tert*-butoxycarbonyl)amino]-3-(5-benzyloxycarbonyl-3-ethoxyisoxazol-4-yl)propionate (1.9 g, 3.6 mmol) and a saturated solution of HCl in diethyl ether (40 mL) was boiled under reflux for 2 h. The formed crystals were collected by
15 filtration, stirred with ethyl acetate, and collected by filtration (0.53 g, 32%): mp 142-144 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 1.32 (t, 3 H), 3.17 (dd, 1 H), 3.25 (dd, 1 H), 4.17-4.32 (m, 3 H), 5.09 (dd, 2 H), 5.39 (s, 2 H), 7.24-7.53 (m, 10 H); MS ($(\text{M} + \text{H})^+$) m/z 425. Anal. calcd, C 59.93, H 5.48, N 6.08; found, C 59.70, H 5.49, N 6.26.

20 Example 9

Ethyl (*RS*)-2-Amino-3-(3-ethoxy-5-ethoxycarbonylisoxazol-4-yl)propionate, Oxalate (Comp. 15)

- A mixture of (*RS*)-2-amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionic acid (2.0 g, 8.2 mmol) and a solution of HCl in ethanol (35 mL) was boiled under reflux for 3 h to give ethyl
25 (*RS*)-2-amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionate. Ethyl (*RS*)-2-Amino-3-(5-carboxy-3-ethoxyisoxazol-4-yl)propionate (0.6 g) was added a dilute solution of NaOH, and the aqueous phase was extracted with ethyl acetate. The organic extracts were washed with brine, dried (MgSO_4), filtered and evaporated to dryness in vacuo. The residue was dissolved in acetone (6 mL) and added a solution of oxalic acid (0.14g, 1.6 mmol) in acetone (6 mL), and
30 the formed precipitate was collected by filtration (110 mg, 10%): mp 159-161 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 1.11 (t, 3 H), 1.32 (t, 3 H), 1.36 (t, 3 H), 3.02 (dd, 1 H), 3.11 (dd, 1 H), 3.98-4.16 (m, 3 H) 4.32 (q, 2 H), 4.37 (q, 2 H); MS ($(\text{M} + \text{H})^+$) m/z 301. Anal. calcd, C 46.15, H 5.69, N 7.18; found, C 46.38, H 5.69, N 7.36.

Example 10

Butyl (RS)-2-amino-3-(5-butoxycarbonyl-3-ethoxyisoxazol-4-yl)propionate, oxalate (Comp. 16)

The compound was obtained in a similar manner as described in Example 9 by using a solution of HCl in butanol. mp 120-121 °C; ¹H NMR (DMSO-*d*₆) δ 0.84 (t, 3 H), 0.92 (t, 3 H), 1.14-1.31 (m, 2 H), 1.31-1.51 (m, 4 H), 1.37 (t, 3 H), 1.62-1.75 (m, 2 H), 3.01 (dd, 1 H), 3.13 (dd, 1 H), 3.98-4.09 (m, 3 H), 4.16-4.36 (m, 4 H); MS ((M+ H)⁺) *m/z* 357. Anal. calcd, C 51.11, H 6.79, N 6.28; found, C 51.06, H 6.82, N 6.35.

Example 11

[4-(2-Amino-3,4-dioxocyclobut-1-en-1-yl)aminomethyl]-3-ethoxyisoxazole-5-carboxylic Acid (Comp. 17)

Ethyl 3-Ethoxy-4-methylisoxazole-5-carboxylate

Acetyl chloride (25 mL, 0.35 mol) was added to EtOH (250 mL) at 0 °C and the solution was stirred at 0 °C for 20 min. A solution of 3-ethoxy-4-methylisoxazole-5-carboxylic acid (WO95/12587,A1) (18 g, 0.10 mol) in EtOH (20 ml) was added and the resulting mixture was boiled under reflux for 4 h. The mixture was cooled, added NaHCO₃ (200 mL) and extracted with diethylether (3 × 300 mL). The organic extracts were dried (MgSO₄) and concentrated in vacuo to afford crude title compound (18 g, 86%).

Ethyl 4-Bromomethyl-3-ethoxyisoxazole-5-carboxylate

Ethyl 3-Ethoxy-4-methylisoxazole-5-carboxylate (18 g, 91 mmol), NBS (17.5 g, 100 mmol), dibenzoyl peroxide (1 g, 4.1 mmol) in tetrachloromethane (500 mL) was boiled under reflux for 16 h. The mixture was cooled, filtered and concentrated in vacuo to afford the crude title compound (24.5 g, 97%).

Ethyl 3-Ethoxy-4-phthalimidomethylisoxazole-5-carboxylate

A solution of ethyl 4-bromomethyl-3-ethoxyisoxazole-5-carboxylate (5 g, 17.9 mmol) in DMF (85 mL) was added to a suspension of potassium phthalimid (3.6 g, 19.7 mmol) in DMF (125 mL) at 90 °C. The resulting mixture was stirred at 90 °C for 40 min, then cooled and concentrated in vacuo. Water (250 mL) was added and the aqueous phase was extracted with diethylether (2 × 200 mL). The organic extracts were dried (MgSO₄) and concentrated in vacuo to give a crude product which was recrystallized (EtOH) to yield the title compound (3.70 g, 60%); mp 93-94 °C.

4-Aminomethyl-3-ethoxyisoxazole-5-carboxylic Acid Hydrochloride

A solution of ethyl 3-ethoxy-4-phthalimidomethylisoxazole-5-carboxylate in 1 M NaOH was boiled under reflux for 45 min. The mixture was cooled, added concd HCl and extracted with

diethylether (3 × 400 ml). The organic extracts were concentrated *in vacuo*, added 1 M HCl (600 mL) and boiled under reflux for 1 h. After cooling the mixture was washed with diethylether (3 × 600 mL) and concentrated in vacuo to give a crude product which was recrystallized (acetic acid) to yield the title compound (1.5 g, 82%): mp 215-216 °C (dec).

5

[4-(2-Amino-3,4-dioxocyclobut-1-en-1-yl)aminomethyl]-3-ethoxyisoxazole-5-carboxylic Acid

To a solution of 4-aminomethyl-3-ethoxyisoxazole-5-carboxylic acid hydrochloride (1.2 g, 5.4 mmol) and 3-amino-4-ethoxy-cyclobut-3-en-1,2-dione (0.60 g, 5.9 mmol) in EtOH (300 ML) was added 1 M NaOH (12 mL). The resulting suspension was stirred at room
10 temperature for 16 h, then concentrated in vacuo, added water (100 mL) and washed with EtOAc (2 × 100 mL). The pH was adjusted to ca. 3 by addition of 1 M HCl. The precipitate was filtered off and recrystallized (water) to afford the title compound as a yellow powder (0.71 g, 47%): mp 236-238 °C (dec). ¹H NMR (DMSO-*d*₆) δ 1.30 (t, 3 H), 4.22 (q, 2 H), 4.68 (bs, 2 H). ¹³C NMR (DMSO-*d*₆) δ 14.41, 35.27, 65.54, 107.16, 159.14, 163.54, 168.71,
15 169.15, 169.73, 183.20, 183.34. MS ((M+ H)⁺) *m/z* 282. Anal. (C₁₁H₁₁N₃O₆, 2.25 H₂O) calcd, C 41.06, H 4.86, N 13.06; found, C 41.16, H 4.46, N 12.96

Pharmacology

20 The compounds of the invention were tested in accordance with the following well recognised and reliable test methods.

[³H]AMPA binding

In this test the affinity of a drug for AMPA receptors is determined by measuring the ability
25 to displace [³H]AMPA from AMPA receptors.

The test was carried out in accordance with a modified version of the method of Honoré, T. and Nielsen, M., *Neurosci.Lett.* 1985, 54, 27-32. The test was carried out in the presence of KSCN. This means that only the [³H]AMPA high affinity binding sites were labelled.

30

The membrane preparations used were obtained in accordance with the method of Ransom, R.W. and Stec, *J.Neurochem.* 1988, 51, 830-836.

The Cortical Wedge Model

35 The Cortical wedge model is a test in which slices of rat brain is examined *in vitro* in order to quantify the effect of ligands at the various Glu-receptors and evaluate the pharmacological profile of the ligands (i.e. agonist/antagonist properties). The test was performed as described

by Harrison, N.L. and Simmonds, M.A. *Br.J.Pharmacol.* 1985, 84, 381-391 as modified according to Wheatley, P.L. *Br.J.Pharmacol.* 1986, 87, 159P.

Table 1. Cortical Wedge

5

Compound	Profile	EC ₅₀ (μM)	pK _i	Receptor subtype
1	Agonist	1.2		AMPA
2	Agonist	4.8		AMPA
(S)-2	Agonist	4.4		AMPA
(R)-2	Antagonist		3.28	AMPA
3	Agonist	40.0		AMPA
8	Agonist	2000		AMPA
10	Agonist	80		AMPA
11	Part. Agonist	325		AMPA
12	Agonist	40		AMPA
13	Antagonist		3.5	NMDA
17	Antagonist		3.3	NMDA

Results

- 10 The compounds were found to be excitatory amino acid (EAA) receptor ligands. Some of the compounds were found to be agonists at the AMPA receptors and other compounds were found to be selective AMPA or NMDA receptor antagonists. The compounds showed activity in the μM range.

Formulation Examples

The pharmaceutical formulations of the invention may be prepared by conventional methods in the art.

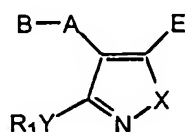
5

For example: Tablets may be prepared by mixing the active ingredient with ordinary adjuvants and/or diluents and subsequently compressing the mixture in a conventional tableting machine. Examples of adjuvants or diluents comprise: corn starch, lactose, talcum, magnesium stearate, gelatine, lactose, gums, and the like. Any other adjuvant or additive
10 colourings, aroma, preservatives etc. may be used provided that they are compatible with the active ingredients.

Solutions for injections may be prepared by solving the active ingredient and possible additives in a part of the vehicle, preferably sterile water, adjusting the solution to the desired
15 volume, sterilisation of the solution and filling in suitable ampules or vials. Any suitable additive conventionally used in the art may be added, such as tonicity agents, preservatives, antioxidants, etc.

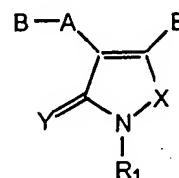
CLAIMS

1. A (3-alkoxyisoxazol-4-yl)-substituted 2-amino carboxylic acid derivative or a sulfur
analogue thereof having general Formula I or II



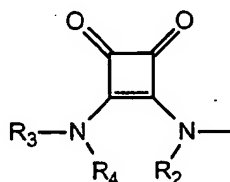
I

or



II

- wherein R_1 is hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, cycloalk(en)yl, cycloalk(en)yl-
10 C_{1-6} alk(en/yn)yl or phenyl- C_{1-6} alk(en/yn)yl the phenyl group being optionally substituted
with CF_3 , halogen, C_{1-6} alkyl or C_{1-6} alkoxy;
A is a bond or a spacer group selected from C_{1-6} alkylene, C_{2-6} alkenylene or C_{2-6} alkynylene,
and cycloalkylene;
B is a group $-CR_a(NR_bR_c)-COOR_s$ wherein $R_a - R_c$ are independently hydrogen or C_{1-6} alkyl,
15 and R_s is defined as R_1 or pivaloyloxymethyl, or B is a group of Formula III



III

- wherein R_2 , R_3 and R_4 are independently selected from the group consisting of
20 a) hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, cycloalk(en)yl, cycloalk(en)yl- C_{1-6}
alk(en/yn)yl, phenyl- C_{1-6} alkyl, thienyl- C_{1-6} alkyl, and
b) C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl in which one or more carbon atoms are replaced by
N, O, and/or S, or
 R_3 and R_4 are connected, thereby forming a C_2-C_6 alkylene, C_2-C_6 alkenylene or C_2-C_6
25 alkynylene group, or
 R_4 and R_2 are connected in order to form a C_1-C_3 alkylene, C_2-C_3 alkenylene or C_2-C_3 alkynyl-
ene group optionally mono- or di-substituted with hydroxy or methyl, or to form CH_2-O-CH_2 ;
E is $COOR_6$, where R_6 is defined as R_s , or E is tetrazol-5-yl, 1,2,4-triazol-3-yl or 1,2,3-
triazol-4-yl;
30 X is O or S; Y is O or S; and
pharmaceutically acceptable salts thereof.

2. A compound of Claim 1, **characterised in**, that it has Formula I.
3. A compound of Claim 1, **characterised in**, that it has Formula II.
- 5 4. A compound of any of Claims 1- 3, **characterised in**, that E is a group COOR₆ wherein R₆ is not hydrogen, preferably C₁₋₆ alkyl phenyl-C₁₋₆, alk(en/yn)yl or pivaloyloxymethyl.
5. A compound of any of Claims 1- 3, **characterised in**, that E is COOH.
- 10 6. A compound of any of Claims 1- 3, **characterised in**, that E is tetrazol-5-yl, 1,2,4-triazol-3-yl or 1,2,3-triazol-4-yl.
7. A compound of any of Claims 1- 6, **characterised in**, that B is a group of the formula CR_aNR_bR_c-COOR₅.
- 15 8. A compound of Claim 7, **characterised in**, that R_b and R_c are hydrogen and R_a is hydrogen or C₁₋₆ alkyl, preferably hydrogen or methyl
9. A compound of Claims 7 or 8, **characterised in**, that R₅ is hydrogen.
- 20 10. A compound of Claims 7 or 8, **characterised in**, that R₅ is not hydrogen, preferably C₁₋₆ alkyl, phenyl-C₁₋₆ alk(en/yn)yl or pivaloyloxymethyl.
11. A compound of any of Claims 1- 6, **characterised in**, that B is a group of the formula
25 III.
12. A compound of Claim 11, **characterised in**, that R₂, R₃ and R₄ are hydrogen or C₁₋₆ alkyl, or R₄ and R₂ are connected in order to form a C₁-C₃ alkylene group.
- 30 13. A compound of Claim 12, **characterised in**, that each of R₂, R₃ and R₄ are hydrogen.
14. A compound of any of Claims 1-13, **characterised in**, that X is oxygen.
15. A compound of any of Claims 1-13, **characterised in**, that X is sulfur.
- 35 16. A compound of any of Claims 1-15, **characterised in**, that Y is oxygen.
17. A compound of any of Claims 1-15, **characterised in**, that Y is sulfur.

18. A compound of any of Claims 1-17, characterised in, that R_1 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl, preferably methyl, ethyl, propyl or propargyl.
- 5 19. A compound of any of Claims 1-18, characterised in, that A is a bond or C_1-C_3 alkylene, preferably methylene.
20. A compound of Claim 2, characterised in, that A is a bond or C_1-C_3 alkylene, B is -
CH(NH₂)-COOH or a group of Formula III wherein each of R_3 , R_4 and R_2 are hydrogen, X and
10 Y are both oxygen, and R_1 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{2-6} alkynyl, preferably methyl, ethyl,
propyl or propargyl.
21. A pharmaceutical composition, characterised in that it comprises a compound of any of
Claims 1 - 20 together with a pharmaceutically acceptable carrier or diluent.
- 15 22. Use of a compound of any of Claims 1 - 20 for preparing a pharmaceutical composition
for treatment of cerebral ischaemia, Huntington's disease, epileptic disorders, Parkinson's
disease, Alzheimer's disease, schizophrenia, pain, depression or anxiety.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00426

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07D 261/12, C07D 261/18, C07D 275/03, C07D 413/04, C07D 417/04, A61K 31/41
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07D, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9512587 A1 (H. LUNDBECK A/S), 11 May 1995 (11.05.95)	1-22
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P,X	J. Med. Chem., Volume 40, 1997, Benny Bang-Andersen et al, "Heteroaryl Analogues of AMPA. Synthesis and Quantitative Structure-Activity Relationships" page 2831 - page 2842	1-22
	--	
A	J. Med. Chem., Volume 39, 1996, Ulf Madsen et al, "Synthesis and Pharmacology of Highly Selective Carboxy and Phosphono Isoxazole Amino Acid AMPA Receptor Antagonists" page 1682 - page 1691	1-22
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☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

09.01.98

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00426

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Am. Chem. Soc., Volume 116, 1994, Shaun D. Abbott et al, "Synthesis and Testing of Heterocyclic Analogues of Diaminopimelic Acid (DAP) as Inhibitors of DAP Dehydrogenase and DAP Epimerase" page 6513 - page 6520 --	1-22
A	Bioorganic & Medicinal Chemistry Letters, Volume 3, No 8, 1993, U. Madsen et al, "Excitatory amino acid receptor antagonists: synthesis and pharmacology of 3-(carboxymethoxy)isoxazoles derived from ampa" page 1649 - page 1654 -- -----	1-22

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INTERNATIONAL SEARCH REPORT
Information on patent family members

02/12/97

International application No.

PCT/DK 97/00426

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		AU 8057994 A	23/05/95
		CA 2175685 A	11/05/95
		CN 1136810 A	27/11/96
		CZ 9601272 A	11/09/96
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		FI 961872 A	03/05/96
		HU 74692 A	28/01/97
		HU 9601167 D	00/00/00
		JP 9504531 T	06/05/97
		NO 961783 A	25/06/96
		NZ 275088 A	22/08/97
		PL 314182 A	02/09/96
		SK 56496 A	04/12/96
		ZA 9408631 A	10/07/95
